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Barrier Function of Stratum Corneum as Studied by Synchrotron X-ray Diffraction

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Introduction

Stratum corneum constituting the outermost layer of the epidermis of skin plays a key role of a major barrier to penetration of molecules through the skin. In the mouse stratum corneum, the lipid lamellar structure characterized by a repeat distance of about 13 nm has been observed in the small angle diffraction and furthermore, the peaks at about 0.38 nm and about 0.42 nm due to the chain packing of lipids have been observed in the wide angle diffraction. In this study, using X-ray diffraction we will show that the lamellar structure is different between the normal stratum corneum and the damaged stratum corneum and furthermore that, when the damaged stratum corneum is immersed in the dilute aqueous suspension of the mixture of ceramide, cholesterol and fatty acid which are dominant component in the lamellar lipid matrix, the damaged stratum corneum is restored structurally to the normal stratum corneum.

Experiment and Results

Sample preparation

The stratum corneum samples were separated from the skin of hairless mouse by treating with trypsin. The damaged samples were prepared as follows. Irritating the mouse epidermis was caused by the closed patch method with 1 wt% sodium dodecylsulfate for 24 h. After removing the patch and leaving for three days, the damaged samples were separated from the skin. The pieces of the samples of about 5 mg were placed in a capillary tube with the diameter of 1 mm. To induce a restoration process, the damaged sample was immersed at room temperature for 48 h in aqueous suspension of the mixture of ceramide 3, cholesterol and stearic acid, which was first resolved in chloroform to mix them, dried and finally dispersed in water with the concentration of 0.05 wt%.

X-ray diffraction

In the normal stratum corneum, we observed diffraction peaks at 13.8 nm, 6.87 nm and 4.59 nm in the small angle region (see Fig.1) which are due to a lipid lamellar structure. These peaks coincide with the previously reported results for mouse stratum corneum [1, 2]. On the other hand, in the damaged stratum corneum, only broad humps remain near the above diffraction peaks in the small angle region (see Fig.1). Furthermore, in the restored stratum corneum, although the dilute suspension itself did not show any diffraction peaks, the recovered diffraction peaks appeared clearly at 13.3 nm,

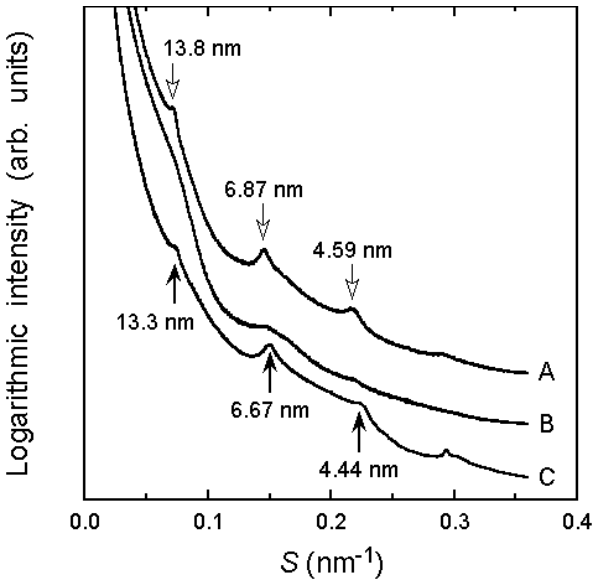

Fig. 1 Small angle X-ray diffraction of the stratum corneum of hairless mouse in the normal stratum corneum (A), the damaged stratum corneum (B) and the restored stratum corneum (C). So as to see easily, the above curves are shifted down successively.

6.67 nm, 4.44 nm and 3.33 nm (see Fig.1) and additionally 3.39 nm that corresponds to the diffraction for cholesterol. It is surprising that the lamellar diffraction peaks of the restored stratum corneum coincide with those of the normal stratum corneum. It is plausible that the slight difference of the lamellar spacing between the normal and restored stratum corneum is due to the difference of the components constituting lamellar lipid matrix.

For the components of the lipids, Bouwstra et al. [5] have pointed out that ceramide 1 plays a crucial role in the in vitro formation of the 12-13 nm lamellar structure in the ceramide/cholesterol mixtures and then, without ceramide 1 only a weak diffraction peak appears around 12 nm. Nevertheless, in the present study by adding only ceramide 3 together with cholesterol and fatty acid to the damaged stratum corneum the diffraction peak at 13 nm reappeared. This should be studied further.

References

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